

Figure 1. Shows different *Candida* spp isolated from pigeon droppings

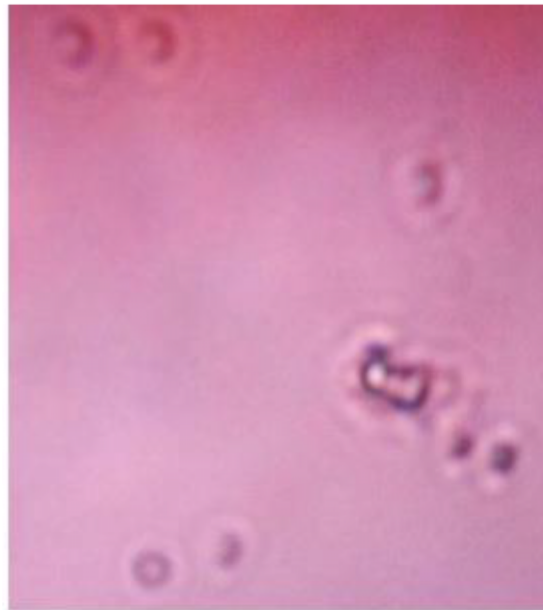


Figure 2. Capsule formation of *Cryptococcus neoformans* using India ink

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**Aspergillosis in Humboldt penguins – susceptibility patterns of clinical and environmental isolates from a Belgian zoo, 2018-2022**

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**Objectives:** Avian aspergillosis causes a heavy burden on birds in captivity, such as Humboldt penguins. In recent years the colony of a Belgian zoo has experienced very high mortality rates and the zoo has already taken several measures to lower the burden. This study was set up to see if the penguins acquire *A. fumigatus* via the environment and if so, if additional measures can be taken to limit the incidence.

**Methods:** A total of 29 clinical strains collected from 2018 to 2022 were included in the study. From April 2021 until January 2022, four samplings have been performed, accounting for every season. A combination of sand, water, nest swabs, and air was analyzed for the presence of (azole-resistant) *A. fumigatus*. In brief, air samples were collected at fixed locations in the

penguin enclosure by impacting 1000 L on an agar plate [malt + chloramphenicol (MC) and MC + tebuconazole (MC + T)]. A total of 100 mL water was collected and 50 mL water was filtered through a 0.22 µm filter and placed on an MC plate, and repeated with the other 50 ml on MC + T. A total of 9 ml 0.1% Tween 20 + 0.85% NaCl was added to 1 g of sand and vortexed for 1 min. Both MC and MC + T plates were incubated with 100 µL of this suspension. All plates were incubated at 48°C ± 1°C for 48 h. The phenotypical resistance pattern of all clinical isolates was determined using the EUCAST method.

**Results:** The phenotypical resistance pattern showed resistance in 7 isolates (24%) with 5 of them showing resistance to posaconazole, one was resistant against voriconazole, isavuconazole, and posaconazole, and 1 showing pan-resistance. No *A. fumigatus* colonies could be detected from water samples, nor from the sand. One *A. fumigatus* isolate was retrieved from the nest swabs. In total 64 *A. fumigatus* colonies were isolated from air samples collected on MC + T medium. All have been subjected to EUCAST microbroth dilution determination and 4 resistant isolates could be detected (all had a MIC value for posaconazole of 0.5 µg/ml and one strain showed additional resistance against isavuconazole with a MIC of 4 µg/ml. Cyp51a sequencing of all resistant strains is ongoing and will give more insight in the molecular mechanisms involved to investigate the potential link with the environment.

**Conclusion:** This study showed high resistance rates in the clinical isolates. Four resistant isolates were found in environmental air samples. Sequencing of the cyp51A gene will give more information on a possible relation between the resistance mechanisms found in the clinical and the environmental isolates. More research should be done to investigate the origin of the resistant isolates in the environment.